

# Dynamics of gene expression under feedback

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Gene expression is a stochastic process governed by the presence of specific transcription factors. Here we study the dynamics of gene expression in the presence of feedback, where a gene regulates its own expression. The nonlinear coupling between input and output of gene expression can generate a dynamics different from simple scenarios such as the Poisson process. This is exemplified by our findings for the time intervals over which genes are transcriptionally active and inactive. We apply our results to the *lac* system in *E. coli*, where parametric inference on experimental data results in a broad distribution of gene activity intervals.

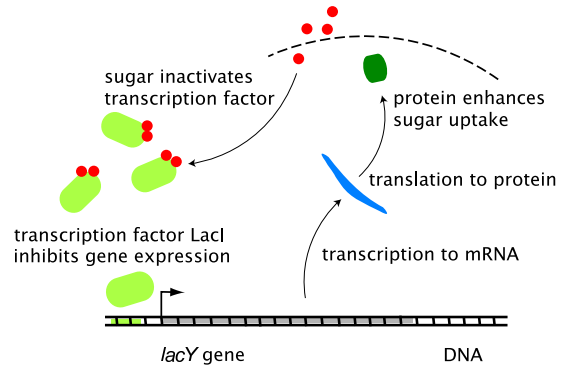
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Gene expression is a dynamic process, which transfers genetic information from DNA to functional molecules such as proteins [1]. This process is controlled by specific proteins, called transcription factors, which bind to DNA typically near the starting point of a gene. Transcription factors can act as enhancers or as repressors of gene transcription by attracting or impeding the molecular machinery which *transcribes* a gene. This machinery, called RNA-polymerase, produces m(essenger)RNA molecules from the DNA template. A single mRNA transcript is later *translated* to several copies of polypeptide chains, which fold into proteins.

Due to the low copy number of the specific molecules typically present in a cell, these processes are intrinsically stochastic. Thus genes can be thought of as ‘toggling’ at random points in time between transcriptionally active and inactive states [2]. One manifestation of this stochasticity is cell-to-cell variations of mRNA and protein numbers in populations of genetically identical cells [3].

The time intervals over which the gene is transcriptionally active can be very short. Frequently, only a single mRNA molecule is produced before an enhancer molecule unbinds again from the regulatory region of a gene, or a repressor molecule binds, causing a change in the transcriptional state of the gene [4]. For short gene-on times, individual mRNA molecules are produced in statistically independent events, which can be modelled by a Poisson process. As a result, fluctuations in mRNA numbers follow Poisson statistics. This picture of gene expression dynamics is frequently referred to as the Poisson scenario [5].

A similarly simple picture emerges if a gene is transcriptionally active long enough to allow for multiple mRNA molecules to be produced. At constant concentration of transcription factors, binding and unbinding of transcription factors to DNA takes place at constant rates. Hence the time intervals over which a gene is active or inactive are distributed exponentially. mRNA molecules are produced while the gene is active, leading



**FIG. 1: Feedback in the *lac* system.** This schematic picture shows transcription and translation of the *lacY* gene to a protein which facilitates the uptake of lactose (a sugar) and its chemical analogues from the environment. Expression of this gene is repressed by the transcription factor LacI. Individual sugar molecules (*e.g.* in the form of allolactose) bind to the transcription factor, rendering the transcription factor less likely to bind to its binding site on DNA. The sugar molecules thus act as effective inducers of *lacY* expression. LacI represses also other genes (*lacA* and *lacZ*), which encode enzymes used to digest lactose. The feedback loop ensures that *lac* genes are repressed in the absence of inducing sugar molecules in the environment.

to bursts in mRNA numbers [5]. Both exponentially distributed gene-on times, and transcriptional bursts have recently been observed experimentally [6, 7].

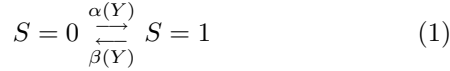
This simple picture of expression dynamics must break down in the presence of feedback, which is the subject of this paper: Direct or indirect coupling between the transcript level of a gene and its transcription rate can introduce a nontrivial dynamics.

An example of feedback is direct autoregulation, which is pervasive in bacteria. Feedback is typically non-linear, since binding of transcription factors to DNA or to other molecules saturates at high concentrations. Feedback can play crucial functional roles, for instance in the *lac* system, which controls the uptake of sugar in bacteria. The gene *lacY* regulates its expression by inactivating its own

repressor (a doubly-negative feedback loop, see Fig. 1).

The consequences of feedback for cell-to-cell variability have been studied both experimentally [8] and theoretically [9, 10, 11]. The analysis of the dynamics in autoregulatory systems, however, has been limited to linear models [12]. In this Letter, we analyze the dynamics of regulatory systems with non-linear feedback kinetics. The dynamic effects of feedback turn out to be particularly marked in the case of the nonlinear doubly-negative feedback, as in the *lac* system, where a broad distribution of gene-on times emerges.

We consider a system consisting of a single gene with transcriptional state  $S(t)$  and number of proteins  $Y(t)$  present in the cell at time  $t$ . The state  $S = 0$  stands for a transcriptionally inactive gene, and  $S = 1$  for an active gene. The dynamics is assumed to be Markovian. To model feedback, both the rate at which the gene goes from the inactive to the active state,  $\alpha(Y)$ , and from the active to the inactive state,  $\beta(Y)$ , can depend on the number of proteins  $Y$ . In the gene-on state, the gene produces mRNA molecules according to a Poisson process with rate of production  $\gamma$ . Each mRNA molecule is translated to a geometrically distributed number of proteins [13], with mean number  $\rho$ , before decaying. The number of proteins  $Y$  decreases at a rate  $\eta$ , which is largely due to dilution by cell division:



where the rate  $\gamma$  is multiplied by  $S$  in the second equation to take into account that mRNA is produced in the active state only. This model assumes a separation of time scales between the mRNA and protein dynamics, with a fast production of proteins from mRNA, and neglects any time delays between a change in the protein level and its effect on the gene activation and inactivation rates. Models that explicitly include the number of mRNA molecules lead to very similar results as tested in numerical simulations.

Fig. 2 shows a sample path of the model indicating both the number of proteins  $Y$  and the transcriptional state of the cell. Fig. 2 also shows the result of a *piecewise deterministic approximation*, which assumes a continuous deterministic increase of the number of proteins at rate  $\gamma\rho - \eta Y(t)$  when the gene is on, and an analogous decrease at rate  $\eta Y(t)$  when the gene is off.

The piecewise deterministic approximation suggests a self-contained model, which retains the transitions between the transcriptional states as the only source of stochasticity. The protein level dynamics  $Y(t)$  in this approximative model thus consists of deterministic exponentially increasing and decreasing paths joined together

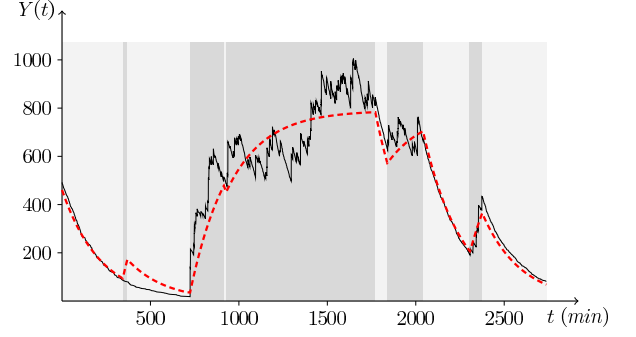


FIG. 2: **Sample path of the model.** The solid curve shows the number of proteins  $Y$  as a function of time. The number of proteins tends to increase when the gene is active ( $S = 1$ , dark background), and decreases when the gene is inactive ( $S = 0$ , light background). The dashed curve shows the piecewise deterministic approximation, see text. The sample path includes an instance where the gene is on for a brief time at ca. 330 minutes, but no transcription takes place. The model parameters were inferred for the *lac* system, see footnote [22]. The lengths of the gene-on intervals can exceed the cell division time (here 216 min) because the protein concentrations are inherited [6].

at randomly positioned switching times of the transcriptional state  $S$ . The switching times themselves depend on the values of  $Y(t)$  because of the feedback. Such models are known as *piecewise deterministic Markov processes* in the mathematical literature [14] and, specifically to describe feedback, *feedback fluid queues* [15]. In the context of gene regulation, such approximations have been considered by Kepler and Elston [9].

The forward Kolmogorov (master) equation of the piecewise deterministic approximation reads

$$\partial_t p_0(y, t) = -\partial_y [(-\eta y) p_0(y, t)] \quad (4)$$

$$+ \beta(y) p_1(y, t) - \alpha(y) p_0(y, t)$$

$$\partial_t p_1(y, t) = -\partial_y [(\gamma\rho - \eta y) p_1(y, t)] \quad (5)$$

$$+ \alpha(y) p_0(y, t) - \beta(y) p_1(y, t),$$

where  $p_i(y, t)$  denotes the probability of the event  $\{S(t) = i, Y(t) = y\}$ . In this approximation,  $y$  has an upper bound  $\Delta := \gamma\rho/\eta$ , which is determined by the balance of protein production and degradation in the gene-on state. Under the biologically reasonable assumption that the rate functions  $\alpha$  and  $\beta$  are bounded away from zero on  $[0, \Delta]$ , the stationary solution to Eqs. (4) and (5) reads

$$\phi_0(y) = \frac{1}{Z} y^{-1} \exp \left[ - \int \left( \frac{\beta(y)}{\gamma\rho - \eta y} - \frac{\alpha(y)}{\eta y} \right) dy \right] \quad (6)$$

$$(\Delta - y) \phi_1(y) = y \phi_0(y), \quad (7)$$

where the subindices again refer to the transcriptional state. The marginal distribution for the number of proteins  $Y$  is given by the sum of the distributions in Eqs. (6)

and (7);

$$f(y) := \phi_0(y) + \phi_1(y) . \quad (8)$$

The constant  $Z$  in Eq. (6) normalizes this distribution.

In the following, we derive the distribution of gene-on times in a stationary process, the analysis of gene-off times being analogous. We define  $T$  as the moment of the first gene inactivation after time  $t_0 = 0$  in a process that was started at  $t = -\infty$ , and consider only those paths of the process that have an activation event taking place immediately after  $t_0$ . The probabilities conditional on the occurrence of such an event are known in the mathematical literature as Palm probabilities [16] and denoted by  $P^0$ . For example, one obtains for the probability that the protein level at the time of activation is less than or equal to  $y$  that [14]

$$P^0(Y(0) \leq y) = Z_0^{-1} \int_0^y \alpha(y) \phi_0(y) dy , \quad (9)$$

where  $Z_0 = \int_0^\Delta \alpha(y) \phi_0(y) dy$  is a normalization constant. The probability that the gene is active longer than for a given time  $\tau$  is then

$$\begin{aligned} P^0(T > \tau) &= E^0 \exp \left( - \int_0^\tau \beta(\tilde{Y}(t)) dt \right) \\ &= Z_0^{-1} \int_0^\Delta \alpha(y) \phi_0(y) \exp \left[ - \int_y^{\Delta - (\Delta - y)e^{-\eta\tau}} \frac{\beta(z)}{\gamma\rho - \eta z} dz \right] dy . \end{aligned} \quad (10) \quad (11)$$

In Eq. (10),  $\tilde{Y}(t) = \Delta - (\Delta - Y(0))e^{-\eta t}$  is an exponentially increasing trajectory with a random initial state  $Y(0)$ , and  $E^0$  is the expectation with respect to  $P^0$ . Eq. (11) follows from Eq. (10) by a change of variable.

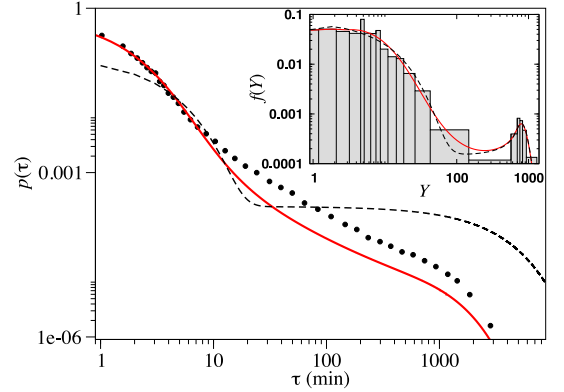
We evaluate these quantities for a concrete example based on the *lac* system. The activation rate  $\alpha$  is taken to be independent of  $Y$  as a first approximation (allowing for  $Y$ -dependent activation rates [17] does not change the general conclusions), whereas the gene inactivation rate  $\beta$  depends of protein level  $Y$ : At high protein levels, the cell takes up sugar molecules from the environment at a high rate, leading to a high steady state concentration of sugar in the cell [8]. This leads to a decreased number of active repressors, as discussed in Fig 1. The gene inactivation rate can be written as the product of the binding rate  $b$  for active repressors and the fraction of active repressors  $\lambda(Y)$ , which is of the Michaelis-Menten form [18]

$$\beta(Y) = b\lambda(Y) = \frac{b}{1 + (A + BY)^2} . \quad (12)$$

Here  $A$  and  $B$  describe the passive and LacY-dependent active uptake of the inducing sugar from the environment respectively [18]. The second power in Eq. (12) arises because two inducers bound to a repressor are needed to

prevent the repressor from binding to DNA [19]. The inactivation rate (12) thus results in a nonlinear regulatory feedback.

Fig. 3 shows the distribution  $p(\tau) = -dP^0(T > \tau)/d\tau$  of gene-on times in the *lac* example. The model parameters were inferred from experimental data of the van Oudenaarden lab [8] as explained in the footnote [22]. Both the result of the piecewise deterministic approximation (solid line), and numeric simulations of the full model (1)–(3) (black dots) exhibit a broad distribution of gene-on intervals, with an exponential cut-off due to saturating inducer concentrations. The small difference between the gene-on distributions between the two models stems from enhancing upward fluctuations at high protein numbers, which are absent in the piecewise deterministic approximation.



**FIG. 3: Distribution of gene on-times in the *lac* system.** The solid curve gives the probability density function, calculated from Eq. (11), for the lengths of time intervals over which the gene is on under the piecewise deterministic assumption. A similarly broad distribution is found in numerical simulations of the model (1)–(3) (black dots). For comparison, the dashed line gives a mixture of two exponential distributions corresponding to a mixture of induced and uninduced cells without feedback. Inset: The histogram shows the distribution of lacY levels measured by the van Oudenaarden lab [8]. Bin widths were chosen to contain equal number of data points. The solid curve gives the corresponding analytical result of Eq. (15), and the dashed curve the result (13) for the exponential model without feedback, with parameters fit to the data in both cases.

These results can be contrasted with a simple model lacking feedback, where gene-on times are exponentially distributed. We consider a mixed population of cells with a fraction  $r$  of cells having gene inactivation rate  $\beta(0)$ , and a fraction  $1 - r$  having rate  $\beta(\Delta)$ . The dashed line in Fig. 3 shows the corresponding gene-on time distribution  $r\beta(0)e^{-\beta(0)\tau} + (1 - r)\beta(\Delta)e^{-\beta(\Delta)\tau}$ . The stationary protein level distributions of the simple mixture of cells reads

$$f_{\text{mix}}(y) = rf_{\beta(0)}(y) + (1 - r)f_{\beta(\Delta)}(y) \quad (13)$$

with

$$f_c(y) = B(\alpha/\eta, c/\eta)^{-1} \Delta^{1-\frac{\alpha+c}{\eta}} y^{\frac{\alpha}{\eta}-1} (\Delta - y)^{\frac{c}{\eta}-1}, \quad (14)$$

where  $B$  denotes the Euler Beta function. This and the corresponding distribution (8) for the piecewise deterministic approximation,

$$f(y) = \frac{\lambda(y)^{\frac{\kappa}{2}}}{Z} y^{\frac{\alpha}{\eta}-1} (\Delta - y)^{\kappa-1} e^{-\kappa(A+B\Delta) \arctan(A+By)}, \quad (15)$$

where  $\kappa = \beta(\Delta)/\eta$ , can both be accurately fitted to the histogram of LacY levels experimentally measured in a population of *E. coli* cells [8], see the inset of Fig. 3. However, the *dynamics* of the transcriptional state is markedly different in the two models, as is evident from the gene-on times in Fig. 3. This shows how dynamic information, now within experimental reach [4], can distinguish between systems with similar statistics of gene expression levels.

In summary, we have shown how feedback shapes the dynamics of regulatory networks. This dynamics also characterizes transitions in multistable regulatory systems. The hysteretic transition from an uninduced state to an induced state of the *lac*-system [8] is accompanied by a divergence of time intervals over which the lacY-gene is transcriptionally active: At low concentrations of the inducing sugar, lacY is active only for short periods of time governed by the rate of repressor binding. At increasing concentrations, the gene on-time distribution broadens (Fig. 3 and Eq. (11)), and finally at high concentrations of the inducing sugar the gene is transcriptionally active most of the time. Viewing the transcriptional state  $S(t)$  of the gene as a continuous field of discrete spin variables in 1D, this behaviour can be seen as a divergence of magnetic domain sizes: Feedback introduces interactions between spins at different times, with a range determined by the protein life-time  $\eta^{-1}$ , which also determines the eventual exponential cut-off in the distribution of gene-on times.

We have focused on a doubly-negative feedback-loop based on the *lac*-system. Positive feedback also generates non-trivial dynamics, however, it is the inverse of gene-off times which turn out to follow a broad distribution. Our analysis is not restricted to systems with direct autoregulation. Regulatory networks typically contain loops generating correlations between different events affecting the transcription of a gene. These correlations are at the heart of deviations from statistical pictures such as the Poisson scenario. An example is feed-forward loops, where the signal from one gene is recombined with a time-delayed copy of itself to produce a simple filter [20]. Feedback loops also play a key role in the control of ionic channels determining the excitability of the heart, where non-exponential distributions of channel-open and -closed times have been observed [21].

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  - [22] The experimental data used in the inset of Fig. 3 gives the lacY distribution at a concentration of 15  $\mu$ M of the inducing sugar TMG. The fluorescence data of Fig. 2b in [8] was rescaled to a steady state protein level of  $Y = 790$  proteins per cell at full induction as determined in [8]. The parameter inference was carried out by convoluting the stationary solutions (13) and (15) with a Gaussian distribution of mean zero and a standard deviation which depends linearly on  $Y$ . This accounts for the inevitable smearing out of the divergences of the stationary protein level distributions by fluctuations of mRNA and protein numbers neglected in the piecewise deterministic approximation, as well as by experimental noise. The inferred parameters of (1)–(3) and (12) for the piecewise deterministic model with feedback are  $\alpha/\eta = 1.59$ ,  $b/\eta = 192$ , and  $B = 0.035$ . The value of  $A$  was found to be negligi-

ble. For the simple mixture of cells, the inferred values are  $\alpha/\eta = 0.9$ ,  $b/\eta = 80$ ,  $b\lambda(\Delta) = 0.08$ , and  $r = 0.35$ . The numerical simulations in Figs. 2 and 3 used  $\eta^{-1}$  determined by the cell cycle time of 216 min given by [18]

for the particular strain of *E. coli* used in [8, 18]. The average number of proteins produced from one mRNA molecule was taken to be 35, as estimated in [18].